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THE CHEMOTHERAPY OF EXPERIMENTAL PNEUMOCOCCUS INFECTION

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The investigations of Morgenroth and his associates on the treatment of experimental pneumococcus infections with various derivatives of quinin and particularly ethylhydrocuprein, coupled with the researches of recent years on the differentiation of pneumococci by serologic methods and the serum treatment of lobar pneumonia, have renewed interest in the possibilities of discovering an efficient chemotherapeutic agent for this infection. A review of the literature on the treatment of pneumonia shows that a large variety of different drugs have been advocated from time to time in treatment on empiric grounds, while very few have been subjected to vigorous laboratory tests with an acceptable technic, for their possible influences on experimental pneumococcus infections. While it is obvious that the treatment of lobar pneumonia presents certain features widely divergent from the pneumococcus bacteriemia of experimental animals, and that certain drugs may exert a favorable influence in the general treatment of pneumonia which are without demonstrable pneumococcidal properties in vitro or in vivo, yet the efforts of the experimental chemotherapeutist must be in the direction of producing drugs possessing high pneumococcidal activity and of sufficiently low toxicity to permit the administration of doses capable of increasing the pneumococcidal activity of the body fluids in the living animals to such degree as will exert definite protective and curative effects against virulent pneumococci in the blood and tissues.

At the present time only derivatives of quinin are known to meet these requirements to any extent worthy of consideration. Advocated in the treatment of pneumonia about 40 years ago by von Jürgensen, Schultz, Aufrecht and Petzold and others, quinin compounds have continued in the favor of a number of practitioners in the treatment of lobar pneumonia, and within recent years Gibson, Galbraith and particularly S. Solis-Cohen, have been warm advocates of these com-

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pounds as holding a place of definite value in the treatment of this infection.

As the investigations of Morgenroth and Levy[1] resulting in the discovery of ethylhydrocuprein (optochin), a synthetic derivative of hydroquinin (methylhydrocuprein) which latter exists in the cinchona bark or can be prepared synthetically, have definitely shown the high pneumococcidal properties of this substance in vitro and in vivo, results which have been generally corroborated by the investigations of Guttmann,[2] Morgenroth and Kauffmann,[3] Levy,[4] Wright,[5] Moore,[6] Cohen, Kolmer and Heist,[7] and others, it would appear that a derivative of quinin superior to ethylhydrocuprein may be discovered, which will exert sufficiently powerful and specific effects as to prove of definite value in the treatment of pneumonia in nontoxic doses.

Attracted by the high germicidal activity in vitro of compounds of mercury for various micro-organisms, calomel and bichlorid of mercury have been advocated empirically by Edelheit, Lepine and others in the treatment of lobar pneumonia, the latter injecting 1 c c of a 1 : 4,000 solution of sublimate directly into the involved lung. These mercurial compounds have not, however, proven of value in the treatment of lobar pneumonia.

EXPERIMENTS WITH NEW MERCURIAL COMPOUNDS

During the past year we have investigated the possible pneumococcidal properties of various new compounds of mercury prepared by Drs. Jay F. Schamberg, George W. Raiziss and John A. Kolmer in the Dermatological Research Laboratories of the Philadelphia Polyclinic, during the course of systematic researches on the chemotherapy of mercurial compounds in experimental trypanosomiasis.[8] We have found that these compounds possess well marked bactericidal properties for pneumococci in the test tube comparable to their bactericidal activity in vitro for staphylococci and typhoid bacilli, as reported by Schamberg, Kolmer and Raiziss.[8] The new compounds of mercury which we have tested for possible protective and curative properties against experimental pneumococcus infections in mice in addition to the ordinary salts of mercury, have included combinations of mercury with naphthol and naphthylanine, phenol, benzidine, aminophenylarsenic acid, and guaiacol.

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[1] Berl. klin. Wchnschr., 1911, 48, p. 1560 and 1970.

[2] Ztschr. f. Immunitätsf., O., 1912, 15, p. 625.

[3] Centralbl. f. Bakteriol., I, Ref., 1912, 54, p. 69.

[4] Berl. klin. Wchnschr., 1912, 49, p. 2486.

[4] On the Pharmacotherapy and Preventive Inoculation Applied to Pneumonia in the African Native, 1915.

[6] Jour. Exper. Med., 1915, 22, p. 269 and 551.

[7] Jour. Infect. Dis., 1917, 20, p. 272 and 313.

[8] Amer. Jour. of Syphilis, 1917, 1, p. 1.

The toxicity of these compounds having been determined, our plan of procedure was to administer graded doses to white mice according to body weight by subcutaneous or intramuscular injection, two hours before the intraperitoneal injection of a lethal dose of pneumococci. We are indebted to Dr. Raiziss for his cooperation in preparing the solutions of these new compounds for administration. In every experiment numerous controls were included.

Throughout our investigation a Type I strain of pneumococcus was employed; before each series of experiments the M. L. D. of a 24-hour broth culture was determined and the strain maintained by animal passage at such virulence that 0.000,001 c c killed within 72 hours.

Attempts to use a smaller dose of culture in order to prolong the lives of the controls by reason of a less severe infection, were generally unsuccessful inasmuch as mice used as controls not infrequently recovered spontaneously and therefore rendered very difficult a correct interpretation of the effects of the compounds under study.

Without dètailing our experiments or presenting tables showing the results, we may state that all of the ordinary soluble salts of mercury and a large number of the new compounds were found by us to be without appreciable effect in prolonging the lives of mice against pneumococcus bacteremia of sufficient severity to kill the controls within 72 hours after infection. Not infrequently the larger doses of these compounds appeared to hasten the deaths of the mice by reason of the summation of the toxic effects of the drug and virulence of the pneumococci; of the new compounds tested, the combination of mercury and guaiacol appeared in some experiments to prolong the lives of mice, but, in general, the results were of a negative character.

EXPERIMENTS IN THE CHEMOSEROTHERAPY OF EXPERIMENTAL PNEUMOCOCCUS INFECTIONS

Investigations in the chemotherapy of pneumococcus infections have indicated that only cinchona compounds or derivatives have any promise as bases and leads in the development of an efficient chemotherapy for this infection. At the present time a derivative of hydroquinin, methylhydrocuprein (Morgenroth's optochin), which occurs in cinchona bark or may be prepared synthetically, and its soluble salt ethylhydrocuprein hydrochlorid, have proven most pneumococcidal in the test tube and in the living animal, although the high toxicity of these drugs has not permitted the administration of sufficient amounts to consistently influence lobar pneumonia. Furthermore, as found by Cohen, Kolmer and Heist,[7] the common compounds of quinin, as hydroquinin, and quinin and urea hydrochlorid, possess a high pneumococcidal activity in vitro and a slight degree of protective power in experimental pneumococcus infections.[9]

Observing that antipneumococcus serum for Type II pneumococci was less efficient than the serum for Type I, Moore[10] studied the influence of a combined optochin and serum treatment for Type II infections among mice, and found that a single dose of optochin suspended in oil and injected subcutaneously, which by itself has practically no protective effect, is capable of increasing the threshold value of Type II serum at least 50 times, and that this effect was proportionately many times greater than a simple summation of the protective and curative effects of the two substances separately. Previous work by Neufeld and Engwer,[11] Engwer[12] and Boehucke[13] also indicated a marked increase of protective and curative effects in mice by combining serum and drug therapy, although it is not clear from their publications that they used homologous strains of pneumococci and antiserums, and, as shown by Moore, an increased effect from the combined drug and serum treatment (chemoserotherapy) cannot be demonstrated unless the antiserum is homologous with the strain of pneumococci producing the infection.

In our studies we have used a virulent Type I strain of pneumococcus and the homologous antiserum combined with ethylhydrocuprein hydrochlorid and several of the commoner compounds of quinin, as the hydrochlorid, quinin and urea hydrochlorid, and hydroquinin; also combinations of the serum with several of the new mercurial compounds prepared in the Dermatological Research Laboratories of the Philadelphia Polyclinic by Schamberg, Raiziss and Kolmer.[8] While a number of these compounds were found without appreciable effect against virulent pneumococcus infections among mice,[8] their pneumococcidal values *in vitro* are very high, and we have considered it worth while to determine whether small doses would appreciably increase the protective and curative value of Type I serum.

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[9] Jour. Infect. Dis., 1917, 20, p. 313.

[10] Jour. Exper. Med., 1915, 22, p. 389.

[11] Berl. klin. Wchnschr., 1912, 49, p. 2381.

[12] Ztschr. f. Hyg. u. Infektionskr., 1913, 73, p. 194.

[13] München. med. Wchnschr., 1913, 60, p. 398.

Each of our experiments were conducted by first determining the minimal lethal dose of 24-hour broth culture of a Type I pneumococcus in 4 days for mice or rats or both, and then the protective power of an antipneumococcus serum in constant dose of 0.05, 0.1 or 0.2 c c against multiple doses of culture during the same interval of time. The animals were then given an intraperitoneal injection of a mixture of culture and antiserum followed immediately with a subcutaneous injection of the drug under study; numerous controls received culture alone; culture and antiserum without drug, culture and drug without antiserum, and drug without culture and antiserum. The results were judged according to the duration of life and the results of bacteriologic examination of the blood of the heart of those animals succumbing during the period of the experiment. As shown by Table 1, we found that 0.1 c c of antiserum (Rockefeller Institute) injected intraperitoneally (equivalent to about 300 c c per 60 kg.) protected mice against 0.1 c c of culture, about 100,000 minimum lethal doses, for 24 hours only, whereas with the same amount of antiserum the subcutaneous injection of 0.002 gm. ethylhydrocuprein hydrochlorid per 100 gm. of mouse (equivalent to 1.2 gm. per 60 kg.) protected for at least 4 days against this dose of culture and for 3 days against 0.2 c c of culture, equal to 200,000 M. D. Other experiments yielded similar results and demonstrated that amounts of ethylhydrocuprein hydrochlorid too small to afford protection against large doses of virulent pneumococi, apreciably increase the protective value of homologous antiserum.

TABLE 1

THE EFFECT OF ETHYLHYDROCUPREIN HYDROCHLORID BY SUBCUTANEOUS INJECTION ON THE PROTECTIVE VALUE OF ANTIPNEUMOCOCCUS SERUM FOR TYPE I PNEUMOCOCCI

Weight in Gm.	Culture in C C	Serum in C C	Drug per 100 Gm. of Body Weight	Results
17	0.05	0.1	—	Died in 48 hours
16	0.1	0.1	—	Died in 48 hours
21	0.2	0.1	—	Died in 24 hours
19	0.1	0.1	0.002	Survived
23	0.2	0.1	0.002	Died in 96 hours
20	0.05	None	0.002	Died in 24 hours

TABLE 2

THE EFFECT OF QUININ AND UREA HYDROCHLORID BY SUBCUTANEOUS INJECTION ON THE PROTECTIVE VALUE OF ANTIPNEUMOCOCCUS SERUM FOR TYPE I PNEUMOCOCCI

Weight in Gm.	Culture in C C	Serum in C C	Drug per 100 Gm. Body Weight	Results	Heart Blood
77	0.5	0.05	—	Died in 24 hours	
48	0.4	0.05	—	Died in 24 hours	Pneumococci
82	0.3	0.05	—	Died in 96 hours	Pneumococci
46	0.2	0.05	—	Survived	
83	0.5	0.05	0.005	Died in 48 hours	Pneumococci
70	0.4	0.05	0.005	Died in 48 hours	Pneumococci
80	0.3	0.05	0.005	Died in 72 hours	
95	0.2	0.05	0.005	Survived	
60	0.4	0.05	0.0025	Died in 24 hours	Pneumococci
119	0.3	0.05	0.0025	Survived	Pneumococci
90	0.2	None	0.005	Died in 24 hours	

We found further that 0.05 c c antiserum intraperitoneally, equivalent to 150 c c per 60 kg., protected a white rat against 0.3 c c culture, 3,000 M.D.D., for 3 days and against 0.2 c c of culture, 2,000 M.D.D., for at least 6 days (Table 2) ; the administration of quinin and urea hydrochlorid in dose of 0.005 gm. per 100 gm. of rat, equivalent to 3 gm. per 60 kg., appeared to exert a slight influence against the higher doses of culture and 0.0025 gm. per 100 gm. of body weight, equivalent to 1.5 gm. per 60 kg., not infrequently prolonged the lives of animals against smaller doses of culture beyond the protection afforded by serum alone. These results are typical of those observed with the other quinin compounds tested, that is, the influence they exerted was usually irregular and feeble.

In the experiment with mice (Table 3) 0.0001 c c of culture alone was fatal within 72 hours; 0.5 c c of antiserum protected against 2,000 fatal doses of culture (0.2 c c) for at least 5 days while the same amount of serum protected against 4,000 doses of culture (0.4 c c) for 2 days and yielded no protection for 5,000 fatal doses of culture (0.5 c c). A second series of mice receiving similar decreasing doses of culture mixed with a constant dose (0.05 c c) of serum followed by the subcutaneous injection of 0.001 gm. of etylhydrocuprein hydrochlorid per 100 gm. of mouse (equivalent to 0.6 gm. per 60 kg.) showed a distinct tendency toward prolonging the lives of mice, while similar doses of quinin and urea hydrochlorid and quinin chlorohydrosulphate also appeared to prolong the lives of several animals although the results were too irregular in all experiments to enable us to express them in more definite terms. All of these drugs alone in the same dosage, 0.001 gm. per 100 gm. of mouse, and injected subcutaneously had no appreciable effect on the large doses of culture employed.

TABLE 3

THE INFLUENCE OF ETHYLHYDROCUPREIN HYDROCHLORID, QUININ AND UREA HYDROCHLORID, AND QUININ CHLOROHYDROSULPHATE BY A SUBCUTANEOUS INJECTION OF 0.001 GM. OF BODY WEIGHT ON THE PROTECTIVE VALUE OF ANTIPNEUMOCOCCUS SERUM AGAINST INCREASING DOSES OF PNEUMOCOCCI TYPE I IN WHITE MICE

Culture in C C	Serum in C C	Ethylhydrocuprein Hydrochlorid	Quinin and Urea Hydrochlorid	Quinin and Chlorohydrosulphate	Result
0.1	—	—	—	—	Died in 24 hours
0.01	—	—	—	—	Died in 24 hours
0.001	— —	—	—	—	Died in 48 hours
0.0001	—	—	—	—	Died in 72 hours
0.5	0.5	—	—	—	Died in 24 hours
0.4	0.5	—	—	—	Died in 72 hours
0.3	0.5	—	—	—	Died in 120 hours
0.2	0.5	—	—	—	Survived 120 hrs.
0.5	0.5	+	—	—	Died in 72 hours
0.4	0.5	+	—	—	Survived 120 hrs.
0.3	0.5	+	—	—	Died in 96 hours
0.2	0.5	+	—	—	Survived 120 hrs.
0.5	0.5	—	+	—	Died in 72 hours
0.4	0.5	—	+	—	Died in 72 hours
0.3	0.5	—	+	—	Died in 120 hours
0.2	0.5	—	+	—	Died in 120 hours
0.5	0.5	—	—	+	Died in 48 hours
0.4	0.5	—	—	+	**Survived** 120 hrs.
0.3	0.5	—	—	+	Died in 72 hours
0.2	0.5	—	—	+	Survived 120 hrs.

TABLE 4

THE INFLUENCE OF ETHYLHYDROCUPREIN HYDROCHLORID, QUININ AND UREA HYDROCHLORID, AND QUININ HYDROBROMID BY SUBCUTANEOUS INJECTION ON THE PROTECTIVE VALUE OF ANTIPNEUMOCOCCUS SERUM AGAINST A CONSTANT LARGE DOSE OF PNEUMOCOCCUS OF TYPE I IN WHITE RATS

Culture in C C	Serum in C C	Cinchona Compound 0.004 Gm. for Each 100 Gm. of Weight	Result
0.1	0.2	—	Died in 48 hours
0.01	0.2	—	Died in 48 hours
0.001	0.2	—	Died in 72 hours
0.0001	0.2	—	Survived 96 hours
0.1	0.2	Ethylhydrocuprein	Died in 96 hours
0.01	0.2	Hydrochlorid	Died in 48 hours
0.001	0.2	....	Died in 24 hours
0.0001	0.2	....	Survived 96 hours
0.1	0.2	....	Died in 48 hours
0.01	0.2	Quinin and	Died in 48 hours
0.001	0.2	Urea Hydrochlorid	Died in 24 hours
0.0001	0.2	....	Survived 96 hours
0.1	0.2	....	Died in 48 hours
0.01	0.2	Quinin	Survived 96 hours
0.001	0.2	Hydrobromid	Died in 24 hours
0.0001	0.2	....	Survived 96 hours

The results of experiments (Table 4) with white rats weighing from 80-130 gm., indicated that 0.2 c c of a serum for Type I pneumococcus protected against 0.1 c c of culture for 24 hours and against 0.0001 c c of culture for more than 96 hours; this strain was of such virulence that 0.0001 c c per 100 gm. of rat killed in 48 hours. Both culture and serum were injected intraperitoneally. Intraperitoneal injection of 0.1 c c of culture per 100 gm. of rat, equivalent to 1,000 M.L.D., with 0.2 c c of serum per 100 gm., equivalent to 120 c c per 60 kg., followed immediately by the subcutaneous injection of 0.004 gm. of ethylhydrocuprein hydrochlorid per 100 gm. of rat, equivalent to 2.4 gm. per 60 kg., protected a rat for 72 hours and another for 24 hours; the serum alone protected for 24 hours but never for a longer period. Quinin and urea hydrochlorid in equal dose in similar experiments exerted no appreciable effects, while quinin hydrobromid in the same amount protected a rat for a period beyond 96 hours. The drugs alone were not toxic and afforded no protection against this large dose of culture.

TABLE 5

THE INFLUENCE OF MERCURIAL COMPOUNDS ADMINISTERED BY SUBCUTANEOUS INJECTION ON THE PROTECTIVE VALUE OF ANTIPNEUMOCOCCUS SERUM FOR TYPE I PNEUMOCOCCI IN WHITE RATS

Culture in C C	Serum in C C	New Mercurial Compound and Dose in Gm. for Each 100 Gm. of Weight	Results
0.1	—	—	Died in 24 hours
0.01	—	—	Died in 24 hours
0.001	—	—	
0.3	0.5	—	Survived 120 hours
0.3	0.5	—	Survived 120 hours
0.3	0.5	—	Died after 120 hours
0.3	0.5	Compound 99—0.01	Died in 72 hours
0.3	0.5	Compound 99—0.0005	Died in 96 hours
0.3	0.5	Compound 99—0.00025	Survived 120 hours
0.3	0.5	Compound 105—0.005	Died in 120 hours
0.3	0.5	Compound 105—0.0005	Died in 96 hours
0.3	0.5	Compound 105—0.00025	Died in 96 hours
0.3	0.5	Compound 42—0.001	Died in 24 hours
0.3	0.5	Compound 42—0.0005	Survived 120 hours
0.3	0.5	Compound 42—0.00025	Died in 96 hours
0.3	0.5	Compound 112—0.001	Survived 120 hours
0.3	0.5	Compound 112—0.0005	Died in 120 hours
0.3	0.5	Compound 112—0.00025	Died in 96 hours
0.3	0.5	Bichl. mercury—0.001	Died in 120 hours
0.3	0.5	Bichl. mercury—0.0005	Died in 96 hours
0.3	0.5	Bichl. mercury—0.00025	Died in 120 hours

The results of one experiment (Table 5) of injecting serum and culture intraperitoneally and graded doses of various known and new preparations of mercury which possess high pneumococcidal powers in vitro subcutaneously, may be given as follows: The culture of Type I pneumococcus employed killed rats weighing from 80-130 gm. in dose of 0.001 c c in 72 hours; 0.05 c c of serum protected against 0.3 c c of culture, 300 M.L.D., for at least 4 days or longer. Bichlorid of mercury administered subcutaneously immediately after the intraperitoneal injection of a mixture of 0.3 c c culture and 0.05 c c serum, did not prolong the lives of any mice; occasionally rats receiving subcutaneous injections of graded doses of several of the new synthetic mercurial compounds lived indefinitely, but the results were always similar to those shown in this table and so irregular and of such a nature as to permit of nothing more than the tentative deduction that several of these compounds in small doses appeared to slightly increase the protective effect of the serum; as previously reported[8] these compounds alone had no appreciable effect on pneumococcus injections among rats and mice.

SUMMARY

The ordinary soluble salts of mercury and numerous new mercurial compounds were found to be without appreciable effect in prolonging the lives of mice infected with a dose of Type I pneumococcus culture lethal within 72 hours.

While systematic researches in the chemotherapy of pneumococcus infections have not been numerous and a large number of different substances cannot be said to have been tried out experimentally, the results of investigations to the present time indicate that derivatives of quinin constitute the best bases or leads in the development of the chemotherapy of pneumococcus infections. It appears that systematic investigations with these compounds is full of promise in the field of chemotherapeutic research of bacterial infections in general and of pneumococcus infections in particular.

The results of our experiments indicate that ethylhydrocuprein hydrochlorid by subcutaneous injection in doses without protective value, usually increase the protective value of antipneumococcus serum Type I in a slight but definite manner in severe infections of mice and rats with homologous pneumococci.

Several of the commoner compounds of quinin, as quinin and urea hydrochlorid, quinin bromid, and quinin chlorohydrosulphate, given subcutaneously in doses without any appreciable influence on severe

and fatal pneumococcus infections, occasionally increase the protective power of antipneumococcus serum but to a lesser extent and less regularly than ethylhydrocuprein hydrochlorid.

Ordinary salts of mercury subcutaneously did not increase the protective value of antipneumococcus serum; several new synthetic compounds of mercury were generally without effect on the protective power of the serum in severe experimental infections.